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(54) Title: DECONTAMINATION USING GUANIDINE THIOCYANATE**(57) Abstract**

A substrate such as medical or veterinary equipment or tissue are decontaminated to remove infectious agents by contacting the substrate with an effective amount of a guanidium compound such as guanidine thiocyanate. Equipment such as surgical or dental instruments are decontaminated by immersion or wetting with a guanidium compound composition, or wiping the surfaces with a material embedded or coated with a guanidium compound composition. Potentially infective tissue and tissue products are typically decontaminated by direct application of a guanidium compound composition to the biological material. One exemplary embodiment employs solutions containing at least about 3 M guanidine thiocyanate. Compositions useful in the practice of the invention may contain adjunct ingredients such as detergents, other wetting agents, stabilizers, and the like. In some embodiments, decontamination methods of the invention include other steps such as heating, sonication, and soaking to enhance the disinfectant effect.

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DECONTAMINATION USING GUANIDINE THIOCYANATE

Technical Field

This invention relates to the decontamination of infectious agents causing Creutzfeldt-Jakob Disease, other transmissible encephalopathies, and the like, using guanidium compounds such as guanidine thiocyanate.

5 Background of the Invention

Creutzfeldt-Jakob Disease (CJD) is a rare but devastating neurodegenerative disease. Although caused by a transmissible agent, clinical symptoms may not become apparent for as long as 30 years after infection. Because white blood cells carry the infectious agent, the possibility that this disease could be transmitted through routine medical and surgical procedures was considered (1). Over the past 10 years England has witnessed an explosive epidemic infection of cows with a similar agent that induces bovine spongiform encephalopathy (BSE). The BSE agent probably originated in processed feed contaminated with the sheep scrapie agent (2) but it is biologically distinct from common scrapie agent strains in sheep (3). It was suggested that variants of the scrapie agent selected in cows might become more virulent for humans than commonly assumed (4). Whereas endemic scrapie infections of sheep have not been linked to human disease, several unusual human CJD cases have now been linked to the bovine infection (5,6). Prion protein (PrP) polymorphisms are sometimes considered critical for infection. However, the single PrP 129 Met-Met polymorphism found in these patients is

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very common (38% of Caucasians), and it is unlikely that this polymorphism is always present in the wide variety of animals that have acquired BSE. There is the unsettling possibility that the incidence of CJD may rise significantly because many individuals, including young people, have been repeatedly exposed to this
5 new and apparently more virulent agent strain.

There is currently no *in vivo* test to detect transmissible encephalopathies during early stages of infection in any species. Nor are there any molecular markers to distinguish agent strains that are more or less virulent for humans. Inadvertent transmissions are therefore problematic. Of especial concern is the
10 likelihood of enhanced virulence of the human adapted BSE agent. Many experiments have shown that once these agents are passaged in a new host they typically become more virulent for that host, yielding more rapidly progressive disease. Thus spreading infections from the known BSE-infected people, as well as from other asymptomatic infected individuals, must be considered. Asymtomatic but
15 infected people have been used as donors for therapeutic tissue and blood products (4). Sensitive tests to detect early infections to prevent iatrogenic spread of disease will ultimately rely on the molecular characteristics of the infectious agent. However these are not resolved and there are two fundamentally different concepts in play. One predominant notion is that a host protein, PrP, converts itself into an
20 infectious protein or prion (7). The alternate view is that the agent is a virus that affects host PrP (4,8,9,10). Neither view is experimentally proven. Although PrP is clearly involved in pathogenesis, purified PrP does not correlate with titer and has failed to transmit infectious disease. On the other hand, although disruption of viral particles reduces CJD titer (11), only candidate viral sequences have been
25 defined (12).

Immediate health-related initiatives must therefore rest on proven experimental approaches to reduce titer and thereby minimize the risk of contracting disease. One practical problem is decontamination of human and animal tissues. Because the agent is infectious at high dilutions, is carried in the reticulo-

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endothelial system, and is highly concentrated in the brain at asymptomatic stages of disease (10,13), general and simple methods to significantly reduce tissue infectivity are needed. These methods should be applicable for routine use on all residual tissues because infectivity cannot be predicted. In a surgical setting,
5 tissue fragments can adhere to operative instruments. Some larger instruments, such as dental drill holders, are not disposable. Even though these instruments can be at some distance from the operative field, they are exposed to tissue microdroplets. Dental work also frequently exposes nerve roots, and thus dental procedures could become a common source for the spread of CJD. Additionally,
10 the expense of discarding specialized surgical instruments after a single use may not be possible in many countries. Finally, previous and proposed slaughter of cattle to reduce BSE exposure in Europe, and the routine removal of brain and spinal cord during butchering, can lead to contamination of large facilities.

Up to the present time three methods have been used to reduce instrument contamination and these generally reduce titer by > 3 logs. They are i) exposure to household bleach (a procedure that can corrode many fine instruments, mechanical parts and stainless steel); ii) prolonged autoclaving (a method not feasible for many large instruments or surfaces); and iii) immersion in concentrated alkaline solutions, *i.e.*, 0.1-1M NaOH. The latter solutions are corrosive not
20 only to instruments and surfaces, but also cause severe skin burns.

Summary of the Invention

It is an object of the invention to provide a new method of decontaminating infectious agents.

It is another object of the invention to provide a decontamination method
25 particularly efficacious for removal of infectious agents causing Creutzfeldt-Jakob Disease and other transmissible encephalopathies from a substrate.

These and other objects are accomplished by the present invention, which provides a method for decontaminating a substrate such as medical or veterinary equipment, tissue, or tissue products to remove infectious agents by contacting the substrate with an effective amount of a guanidium compound such as 5 guanidine thiocyanate. In a typical embodiment, medical or veterinary equipment such as surgical or dental instruments are decontaminated by immersing or wetting the equipment in a composition containing a guanidium compound, or by wiping the surfaces of the equipment with a material embedded or coated with a guanidium compound composition. One embodiment employs solutions containing 10 at least about 3 M guanidine thiocyanate. In another embodiment, potentially infective tissue or tissue products are decontaminated by contacting them with a composition containing from about 0.5 to about 3.5 M guanidium compound.

Methods of the invention may include other steps such as sonication and/or heating (including autoclaving) and/or soaking articles in the presence of a 15 guanidium compound, as well as other steps incorporating other methods of disinfection and cleaning such as treatment with chlorine dioxide and other germ-killing compositions.

Guanidine thiocyanate, guanidine hydrochloride, and the like guanidium compound compositions used in the practice of the invention may contain other 20 ingredients such as detergents, wetting agents, stabilizers and the like in the formulations.

Detailed Description of the Invention

Broadly speaking, this invention provides a method for decontaminating a substrate to remove infectious agents by contacting the substrate with an effective 25 amount of a guanidium compound. Substrates include any type of surface or

carrier which could provide a locus for the accumulation of viruses, bacteria, fungi, spores and the like disease-causing microorganisms and pathogens. Typical substrates include, but are not limited to, medical and veterinary equipment, especially hardware such as surgical and dental instruments, as well as potentially 5 infective tissue and tissue products.

In the practice of the invention, a substrate to be decontaminated is contacted with an effective amount of a guanidium compound (herein occasionally referred to as Gdn). Guanidium compounds include guanidine hydrochloride, guanidine thiocyanate, related compounds, including guanidium-containing 10 compounds and derivatives, and mixtures thereof. By "guanidine thiocyanate" is meant guanidine thiocyanate (GdnSCN), active derivatives of guanidine thiocyanate, guanidine isothiocyanate, active derivatives of guanidine isothiocyanate, and mixtures thereof. Correspondingly, by "guanidine hydrochloride" is meant guanidine hydrochloride (GdnHCl), active derivatives of guanidine hydrochloride, and mixtures thereof. In preferred embodiments, the substrate is contacted with a 15 composition containing GdnSCN; liquid compositions are particularly preferred. Liquid compositions of the invention include any type that will dissolve, suspend, or otherwise disperse the active ingredient such as solutions, suspensions, gels, and the like. Aqueous GdnSCN, GdnHCl, or GdnSCN/GdnHCl solutions are 20 employed in many embodiments. Though lower concentrations may be employed where substrates are soaked in guanidium compositions for extended periods, typical concentrations range between about 2.5 and 6 M. As will be illustrated more fully in the next section, compositions containing at least about 2.5 M, preferably greater than 3.8 M, GdnSCN are useful for certain applications.

25 For potentially infective biological materials such as tissue or tissue products, including blood, blood products (including serum proteins), and organs involved in transplants, lower concentrations, i.e., from about 0.5 M to about 3 M, are employed in typical embodiments. In some embodiments involving decontamination of biological material such as therapeutic tissue and blood

products, potentially transmissible agents are removed prior to extraction of medically active compounds by contact with guanidium solutions. The decontamination solution is then removed from the tissue.

5 Compositions useful in the methods of the invention may contain other ingredients in the formulation, preferably those that are inert in the sense of not bringing about a significant deactivation of the active ingredient. These include other disinfectants or detergents that have disinfectant properties that augment the active ingredient of the invention; stabilizers that prolong the shelf-life of products containing GdnHCl and/or GdnSCN; wetting agents and detergents that enhance or assist application and/or penetration, and/or hold active ingredient in contact with the substrate; and the like. In an embodiment illustrated hereafter, a GdnSCN composition containing sarkosyl was employed.

15 The substrate is typically contacted with GdnSCN and/or GdnHCl for such time under such conditions to effect decontamination of the surface. In most embodiments, a statistically significant decrease in viral, bacterial, and/or fungal titer, preferably by ≥ 3 logs, from the undecontaminated substrate is observed. In an embodiment described in the Examples that follow, animal assays are employed to assess the extent of decontamination. The substrate may be immersed 20 in or wet with the GdnSCN or other guanidium composition, or wiped with a material embedded or coated with a GdnSCN or other guanidium composition.

Methods of the invention employing GdnSCN and/or GdnHCl may be combined with other methods of sterilization, cleansing, and sanitizing. For example, some embodiments, particularly those involving the disinfection and 25 sterilization of surgical and dental instruments, may involve a sonication and/or at least one heating step. Heating to at least about 55°C, and in some cases to at least about 75°C, is preferred. Instruments may be autoclaved in the presence (if in accord with E.P.A. requirements) or absence of GdnSCN and/or GdnHCl before or after contact with it. In one embodiment, instruments immersed in a

GdnSCN composition are autoclaved to achieve effective decontamination of particularly recalcitrant and/or dangerous pathogens. Medical or veterinary hardware may be sonicated before or after treatment with GdnSCN, or sonicated in its presence such as in an ultrasonic cleaning apparatus; this is particularly 5 advantageous where there is tissue adhering to the equipment or where vigorous washing is not possible. Washing and other disinfectants such as treatment with Clorox® or chlorine dioxide, hydrogen peroxide, base, acid, and the like may be used in conjunction with GdnSCN and/or GdnHCl use.

It is an advantage of the invention that GdnSCN and/or GdnHCl is/are 10 particularly efficacious for removal of infectious agents causing Creutzfeldt-Jakob Disease and other transmissible encephalopathies from a substrate, as well as other resistant pathogens such as hepatitis B. They are also likely to be effective for other resistant pathogens. In embodiments involving the treatment of tissue or tissue products, the invention provides for extraction or separation of medically 15 relevant biological material free of transmissible agents.

The following are presented to further illustrate and explain the present invention and should not be taken as limiting in any regard.

Examples

20 In studies to uncover essential molecular components of the CJD agent it was found found that concentrations of GdnHCl as low as 2.5M effectively disrupted the nucleic acids and capsid proteins of viral particles. Concomitantly, CJD titer was reduced by ~ 3 logs (99.7%), whereas solubilization and separation of PrP from sedimenting infectious particles did not reduce titer (11). It was 25 therefore suggested that GdnHCl treatment might be used to reduce contamination and experiments were initiated to validate this approach. Others subsequently hypothesized that GdnHCl might be useful for dental instrument decontamination

(14). More disruptive GdnSCN solutions could result in even greater levels of agent inactivation and therefore verified this experimentally.

To simplify the method for clinical settings, a commercially available solution of 4M GdnSCN was used (RNA lysis buffer #40082, Perkin Elmer).

5 This solution has a similar composition as the one we use to efficiently extract nucleic acids from more purified CJD preparations (12). It conveniently contains a detergent that can aid in tissue penetration and disruption, and also has a reasonable shelf life of > 1 year. Moreover, this solution can be easily modified to further enhance decontamination. Moderate heat treatment was also tested to find

10 if agent inactivation could be augmented. The temperatures tested are easily achieved with old instrument sterilizers in dentists offices. Brain tissue from a long established CJD hamster model (serial passage 31, strain SY) was chosen because it yields reproducible titers. The very high lipid composition and complexity of brain can compromise inactivating procedures and therefore whole brain

15 was chosen as a difficult inactivation target. Experimental groups of six hamsters each were injected with: 1) CJD homogenates in saline without inactivation, 2) CJD saline homogenates treated at 75°C for 25 min, 3) CJD homogenates lysed in GdnSCN and 4) CJD GdnSCN homogenates treated at 75°C for 25 min.

To maximize viral input each half of a CJD infected brain was directly

20 homogenized in either normal saline or GdnSCN lysis buffer (10% w/v). A 100-ml aliquot of each respective homogenate was heated in a 1.5 ml polypropylene tube in a PCR machine while the parallel homogenates were kept at 22°C. Each of the four homogenates were then diluted to 0.5 mg brain/ml and 50 ml was inoculated intracerebrally per hamster (11). The large dilution was calculated 1) to preclude any toxic effects of GdnSCN, 2) to yield incubation time titers in the linear range for saline homogenates (20), and 3) to approximate end-point LD₅₀ determinations for GdnSCN samples.

As summarized in Table 1 (below), hamsters inoculated with CJD brain in saline developed clinical disease by 166 days. There was a small (~ 3 fold or 0.55 logs) but statistically significant decrease in titer with moderate heating in saline. Typical CJD changes (10) were verified histologically in both saline groups. A far more substantial reduction in titer was found in GdnSCN treated parallel samples. Indeed, none of these animals showed any signs of disease at > 350 days post inoculation. With GdnSCN the titer was reduced by ~ 5 logs (2.3×10^8 /gm to < 10^3 /gm). Because the GdnSCN treatment alone was so effective, the effect of heating GdnSCN homogenates to increased inactivation could not be assessed. Nonetheless, the saline controls indicate that heating at 75°C or higher should yield even more effective inactivations, although toxic fumes at 75°C could be released.

Additional experiments on infectious concentrates have shown heating at 55°C further enhances disruption by molecular analyses, and heat increase penetration into mechanical lubricants (15). Exposure of stainless steel surgical instruments (Dumont tweezers) for 24 hours to 4M GdnSCN, moreover, showed no corrosive effects. Therefore nondisposable surgical instruments may be significantly decontaminated by immersion and sonication in the above GdnSCN solution (GdnSCN wipes for steel surfaces can be incinerated). These treatments may have universal application for the inactivation of many other infectious agents, including non-enveloped viruses that are relatively resistant to inactivation such as hepatitis B. A recent outbreak of Hepatitis B was traced to incompletely sterilized EEG needles (see below) and perhaps could have been avoided with the above precautions.

The outlined protocol is also based on the inability to find any reconstitution of CJD infectivity when more purified infectious preparations were treated with less concentrated and disruptive GdnHCl solutions (11). After dilution of GdnHCl, the reduction in titer remained. Thus washing trace GdnSCN should have few risks. Using similar GdnHCl treatment and dilution, another laboratory

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reported that PrP rapidly refolded into an "infectious conformation" as assessed by gel assays. However, there was no data showing infectious titer was restored (16). Moreover, similar refolding experiments have been irreproducible, with PrP conversion ascribed to an artifact of incompletely denatured aggregates (17).

5 Older studies have likewise shown reductions in scrapie titer with other Gdn solutions, but the application of such treatments for decontaminating instruments and surfaces have not been previously considered.

Use of a standard solution and protocol should help eliminate variable inactivations. Although somewhat different sensitivities to the same chemical and 10 physical treatments have been reported with different agent strains (e.g., hamster adapted scrapie and CJD), all preparations are highly sensitive to >3M GdnSCN. Additionally, minor methodological and preparation differences can often account for vastly different claims about the resistance of these agents. The efficacy ascribed to these agents should be viewed in the framework of conventional viral 15 studies where absolute inactivations are rarely achieved. Indeed, glutaraldehyde-treated HIV material can be infectious (15), and after dry heat treatment of tissue products at 80°C, the risk of Hepatitis C infections can still be as high as 10% (18). These two enveloped viruses are relatively sensitive in the viral spectrum. Furthermore, Hepatitis B, a more resistant virus, was not inactivated when dry 20 heat sterilizers were used to decontaminate reusable subcutaneous EEG electrodes (19).

Incomplete inactivations may be due, at least in part, to inadequate tissue penetration. Penetration should be significantly enhanced with strongly disruptive GdnSCN. Nonetheless, methods of the invention are disclosed with the 25 recognition that absolute inactivations of CJD-like agents or other resistant viruses can not yet be guaranteed. Indeed, it is possible that the incomplete but still harsh rendering process used in the production of scrapie-infected feed itself selected for more resistant agent variants causing BSE. Large BSE-infected cattle are currently being sterilized by cremation, and some cow remains have already been dumped in

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open pits in northern Scotland. There is also the possibility that some remains may be incompletely ashed, and it may be prudent now to take added new steps to prevent the spread of residual, and perhaps more resistant agent variants to wild animals. Treatment of incompletely ashed or partially inactivated remains with 5 GdnSCN derivatives could help prevent further propagation of evolving virulent strains.

Table 1

<u>Group</u>	<u>Days signs (SEM)</u>	<u>Titer/gm (SEM)</u>
1) CJD-saline	166.2 (1)	2.3×10^8 (2.1×10^7)
10 2) CJD-saline & heat	180.2 (0.5)	6.5×10^7 (2.8×10^6)
3) CJD-GdnSCN	> 350	$\leq 10^3$
4) CJD-GdnSCN & heat	> 350	$\leq 10^3$

TABLE I: Days incubation to clinical signs of disease and titer per gram of brain for each experimental group. Two-tailed t-test showed significant reduction in homogenate titer after moderate heating in saline (p < 10-6), with far more inactivation after GdnSCN (longer incubation time). Methods: To maximize viral input each half of a CJD infected brain was directly homogenized in either normal saline or GdnSCN lysis buffer (10% w/v). A 100 ml aliquot of each respective homogenate was heated in a 1.5 ml polypropylene tube in a PCR machine while 15 the parallel homogenates were kept at 22 °C. Each of the four homogenates were then diluted to 0.5 mg brain/ml and 50 µl was inoculated intracerebrally per hamster (11). The large dilution was calculated 1) to preclude any toxic effects of GdnSCN, 2) to yield incubation time titers in the linear range for saline homogenates (20) and 3) to approximate end-point LD50 determinations for GdnSCN 20 samples. Titer calculations are based on the conservative estimate that 1 infectious unit (IU) will produce clinical signs at 294 days, and thus end-point determinations at 600 days yield a fraction of 1 IU, e.g., 1 of 5 animals with disease at 600 days 25 is equivalent to 0.2 IU in the inoculum.

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The above description is for the purpose of teaching the person of ordinary skill in the art how to practice the present invention, and it is not intended to detail all those obvious modifications and variations of it which will become apparent to the skilled worker upon reading the description. It is intended, however, that all such obvious modifications and variations be included within the scope of the present invention, which is defined by the following claims. The claims are intended to cover the claimed components and steps in any sequence which is effective to meet the objectives there intended, unless the context specifically indicates the contrary.

The invention was made with partial support from Grants NS12674 and NS34569 from the National Institute of Health. The government has certain rights in the invention.

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The above papers are hereby incorporated herein in their entireties by reference.

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Claims

1. A method for decontaminating a substrate to remove infectious agents comprising contacting the substrate with an effective amount of guanidine thiocyanate.
2. A method according to claim 1 wherein the substrate comprises the surfaces of surgical or dental instruments.
3. A method according to claim 2 comprising immersing the instruments in a liquid composition containing guanidine thiocyanate.
4. A method according to claim 3 wherein the composition is a solution containing at least about 4 M guanidine thiocyanate.
5. A method according to claim 3 further comprising the step of sonicating the instruments when they are immersed in the guanidine thiocyanate composition.
6. A method according to claim 3 further comprising at least one autoclaving step.
7. A method according to claim 6 wherein the immersed instruments are autoclaved in the guanidine thiocyanate composition.
8. A method according to claim 1 wherein the substrate is wiped with a material embedded or coated with a guanidine thiocyanate composition.
9. A method according to claim 8 wherein the substrate is wiped with a detergent solution after wiping with the guanidine thiocyanate composition.
10. A method according to claim 1 wherein the substrate is tissue which is decontaminated by pouring an excess guanidine thiocyanate composition on the tissue.

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11. A method of decontaminating equipment, tissues or tissue samples to remove infectious agents comprising contacting said equipment, tissues or tissue samples with a composition containing an effective amount of guanidine thiocyanate.
12. A method according to claim 11 wherein the composition is a solution.
13. A method according to claim 12 wherein the composition contains at least about 4 M guanidine thiocyanate.
14. A method according to claim 13 wherein the equipment comprises surgical or dental instruments that are decontaminated by immersing in the guanidine thiocyanate composition.
15. A method according to claim 11 wherein the equipment or tissues are wiped with a material coated or embedded with the guanidine thiocyanate composition.
16. A method according to claim 11 further comprising the step of heating the equipment, tissues or tissue samples in the presence of the guanidine thiocyanate composition.
17. A method for decontaminating the surfaces of medical equipment to remove infectious agents comprising contacting the surfaces with a liquid composition containing at least about 3 M guanidine thiocyanate.
18. A method according to claim 18 further comprising the step of heating the liquid composition when in contact with the medical equipment surface.
19. A method according to claim 19 wherein the composition is heated to at least about 55°C.

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20. A method for decontaminating a biological sample to remove transmissible agents comprising contacting the sample with a composition containing an effective amount of guanidine thiocyanate.
21. A method according to claim 21 wherein the sample is further extracted to obtain medically active compounds.

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AMENDED CLAIMS

[received by the International Bureau on 3 June 1998 (03.06.98);
original claims 18, 19 and 21 amended;
remaining claims unchanged (2 pages)]

11. A method of decontaminating equipment, tissues or tissue samples to remove infectious agents comprising contacting said equipment, tissues or tissue samples with a composition containing an effective amount of guanidine thiocyanate.
12. A method according to claim 11 wherein the composition is a solution.
13. A method according to claim 12 wherein the composition contains at least about 4 M guanidine thiocyanate.
14. A method according to claim 13 wherein the equipment comprises surgical or dental instruments that are decontaminated by immersing in the guanidine thiocyanate composition.
15. A method according to claim 11 wherein the equipment or tissues are wiped with a material coated or embedded with the guanidine thiocyanate composition.
16. A method according to claim 11 further comprising the step of heating the equipment, tissues or tissue samples in the presence of the guanidine thiocyanate composition.
17. A method for decontaminating the surfaces of medical equipment to remove infectious agents comprising contacting the surfaces with a liquid composition containing at least about 3 M guanidine thiocyanate.
18. A method according to claim 17 further comprising the step of heating the liquid composition when in contact with the medical equipment surface.
19. A method according to claim 18 wherein the composition is heated to at least about 55°C.

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20. A method for decontaminating a biological sample to remove transmissible agents comprising contacting the sample with a composition containing an effective amount of guanidine thiocyanate.
21. A method according to claim 20 wherein the sample is further extracted to obtain medically active compounds.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/01324

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) A01N 25/00; A61L 2/18

US CL. 422/28, 20, 292

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. 422/28, 20, 292

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

search terms: guanid?, decontam?, steriliz?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,537,746 A (OGUNBIYI et al) 27 August 1985, especially column 3, lines 13-53.	1-17, 20
X	US 5,000,867 A (HEINHUIS-WALTHER et al) 19 March 1991, especially column 1, lines 45-59.	1-17, 20
X	OKEN et al. Letter to the Editor. Journal of Dental Research. 1995, Vol.74, page 1836.	1-17, 20
X	MANUELIDIS et al. Viral particles are required for infection in neurodegenerative Creutzfeldt-Jakob disease. Proceedings of the National Academy of Sciences, USA. May 1995, Vol.92, pages 5124-5128, especially page 5128.	1-17, 20

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

24 MARCH 1998

Date of mailing of the international search report

21 APR 1998

Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01324

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: 18, 19, and 21 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Each claim has been written as depending from itself, thus making the said claims unsearchable.

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

